Annals of Applied Biology ISSN 0003-4746

RESEARCH ARTICLE

Genetic diversity in morphological characters and phenolic acids content resulting from an interspecific cross between eggplant, *Solanum melongena*, and its wild ancestor (*S. incanum*)

J. Prohens[1], B.D. Whitaker[2], M. Plazas[1], S. Vilanova[1], M. Hurtado[1], M. Blasco[3], P. Gramazio[1] & J.R. Stommel[4]

1 Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain
2 Food Quality Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, USDA, Beltsville, MD, USA
3 Instituto Valenciano de Investigaciones Agrarias, Apartado Oficial, Moncada, Spain
4 Genetic Improvement of Fruits and Vegetables Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, USDA, Beltsville, MD, USA

Keywords
Breeding; characterisation; chlorogenic acid; functional quality; interspecific hybridisation; introgression.

Correspondence
Prof. J. Prohens, Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain.
Email: jprohens@btc.upv.es

Received: 28 September 2012; revised version accepted: 19 December 2012.

doi:10.1111/aab.12017

Abstract

Solanum incanum, the wild ancestor of eggplant, *Solanum melongena*, has been considered as a source of variation for high content of phenolic acid conjugates in breeding programmes aimed at improving the functional quality of eggplant. We have evaluated the morphological and phenolic acids content in an interspecific family including *S. incanum* (P1), *S. melongena* (P2), their interspecific hybrid (F1), progeny from the selfing of the F1 (F2) and the backcross of the F1 to P2 (BC1P2). Many morphological differences were found between parents, while the F1 was intermediate for most traits. However, F1 plants were taller and pricklier and presented higher fruit flesh browning than any of the parents. F2 and BC1P2 were morphologically highly variable and the results obtained suggest that a rapid recovery of the characteristic combination of *S. melongena* traits can be achieved in a few backcross generations. Segregation for prickliness was found to be compatible with simple genetic control, prickliness being dominant over non-prickliness. A total of 16 phenolic acid conjugates were studied, of which chlorogenic acid (5-*O*-(*E*)-caffeoylquinic acid) was the most common compound in all samples, averaging 77.8% of all hydroxycinnamic acid derivatives. Contents of total phenolic acid conjugates were much higher in *S. incanum* than in *S. melongena* fruit flesh, and no major differences were found in the profile of phenolic acids among parents. The interspecific hybrid (F1) was intermediate between the two parents in phenolic acids content. Non-segregating generations presented considerable variation in phenolic acids content, but the range of variation was wider in segregating F2 and BC1P2 generations. Additive genetic effects were the most important in explaining the results obtained for the phenolic acids content. A number of BC1P2 plants presented a good combination of phenolic acids content and fruit weight or flesh browning. Overall, the results demonstrate that improvement of functional quality in *S. melongena* can be obtained using *S. incanum* as a donor of alleles for high phenolic acids content.

Introduction

Eggplant, *Solanum melongena* L., is one of the vegetables ranked highest in total antioxidant capacity, which is attributed to its high content of phenolics (Cao *et al.*, 1996; Hanson *et al.*, 2006; Okmen *et al.*, 2009; Akanitapichat *et al.*, 2010; Lo Scalzo *et al.*, 2010). The main phenolics in eggplant fruit are hydroxycinnamic acid (HCA) conjugates, predominantly chlorogenic acid

(5-*O*-(*E*)-caffeoylquinic acid; 5-CQA), which constitutes up to 95% of the total phenolic acids present in the fruit flesh (Stommel & Whitaker, 2003; Whitaker & Stommel, 2003; Singh *et al*., 2009). In addition, purple pigmented eggplant accessions contain anthocyanins derived from delphinidin, in the fruit epidermis (Azuma *et al*., 2008). These anthocyanins also have potent antioxidant activity (Azuma *et al*., 2008), but their concentration in eggplant fruit is relatively small compared with HCA conjugates present in the flesh.

Chlorogenic acid has many beneficial properties for human health, including free radical scavenging, anticarcinogenic, cardioprotective, anti-obesity, anti-inflammatory, analgesic and antipyretic activities, and it also regulates the level of glucose in blood (Sawa *et al*., 1998; Triantis *et al*., 2005; Lee & Zhu, 2006; dos Santos *et al*., 2006; Kwon *et al*., 2008; Cho *et al*., 2010; Dai & Mumper, 2010; Coman *et al*., 2012). Potential health benefits, including anti-HIV activity, have also been identified for other minor HCA conjugates in fruits of eggplant and wild relatives (McDougall *et al*., 1998; Ma *et al*., 2011). Furthermore, chlorogenic acid and other HCA conjugates present in eggplant are highly stable (Friedman & Jürgens, 2000; Lo Scalzo *et al*., 2010). In fact, it has been reported that the levels of bioavailable phenolic acids in eggplant subjected to different cooking treatments are even higher than those present in the raw product (Lo Scalzo *et al*., 2010).

Given the increasing interest in vegetables with a higher content of functional compounds, the development of eggplant cultivars with increased amounts of HCAs, in general, and of chlorogenic acid in particular, in the fruit flesh is warranted (Stommel & Whitaker, 2003; Prohens *et al*., 2007). However, oxidation of phenolics causes browning of the fruit flesh after cutting and exposure to air, which is an undesired trait (Queiroz *et al*., 2008). Therefore, selection against fruit flesh browning in breeding programmes has resulted in the indirect selection for low phenolics content. As a consequence, modern varieties of eggplant often have a lower concentration of phenolic acids in comparison with traditional local varieties (Prohens *et al*., 2007).

In order to be efficient, breeding programmes aimed at increasing the phenolics content of eggplant fruit require germplasm resources with sufficiently high levels of variation for these compounds. Although several fold differences in the content of phenolic acid conjugates within the cultivated species have been reported (Stommel & Whitaker, 2003; Hanson *et al*., 2006; Prohens *et al*., 2007; Okmen *et al*., 2009; Akanitapichat *et al*., 2010), the highest content has been found in wild relatives of eggplant, including *Solanum incanum* L. (Stommel & Whitaker, 2003; Ma *et al*., 2010, 2011). *S. incanum* is the wild ancestor of eggplant (Lester & Hasan, 1991; Meyer *et al*., 2012) and hybrids with the cultivated eggplant *S. melongena* are easily obtained. Those hybrids are completely fertile and present regular meiosis and segregation of markers (Lester & Hasan, 1991; Vilanova *et al*., 2010; Daunay, 2012). Therefore, *S. incanum* represents a viable source of variation for phenolics content that can be effectively introduced into breeding programmes aimed at increasing eggplant fruit phenolics content. In addition, *S. incanum* presents other traits of interest that may be useful in eggplant breeding. These attributes include resistance to *Fusarium oxysporum* and tolerance to abiotic stresses such as drought (Yamakawa & Mochizuki, 1979; Lester & Hasan, 1991).

One of the disadvantages of including *S. incanum* as source of variation for phenolics content in an eggplant breeding programme is that many unfavourable traits from the agronomic and commercial point of view are present in this wild species, including the presence of prickles, small fruit, and lack of anthocyanins in the skin (Lester & Hasan, 1991). These traits have to be selected against and removed in the backcross breeding programme, which may be difficult depending on the number of genes and genetic control involved in each trait (Fita *et al*., 2010). Furthermore, the genes of interest in the wild species that are responsible for the traits of interest might be linked to unfavourable genes (linkage drag). Therefore, as in other breeding programmes in eggplant involving interspecific hybridisation (Prohens *et al*., 2012), breeding for high phenolics content in eggplant must take into account not only phenolics content in segregating generations between *S. melongena* and *S. incanum* but also morphological and agronomic traits of interest.

Here, we evaluate the inheritance of morphological attributes that contribute to fruit quality, as well as the content of phenolic acid conjugates, in segregating F2 and backcross generations resulting from an interspecific cross between *S. incanum* and *S. melongena*. The objective was to obtain information of relevance for improvement of the functional quality of eggplant as well as to assess the feasibility of developing materials of commercial interest from interspecific crossings between *S. melongena* and its wild relative *S. incanum*.

Materials and methods

Plant material

One interspecific family consisting of one parental accession of *S. incanum* (MM577; P1), one parental accession of *S. melongena* (ANS26; P2), the interspecific hybrid between them (ANS26 × MM577; F1), the selfed hybrid (F1 × F1; F2) and the first generation hybrid backcrossed to the *S. melongena* parent (ANS26 × F1; BC1P2). The

S. incanum parent originated in Israel, and its fruit are small and green; the *S. melongena* parent is a landrace from Spain with large purple fruit. Six plants were evaluated for each of the P1, P2 and F1 non-segregating generations. For the F2 and BC1P2 generations, 41 and 64 plants were evaluated, respectively. Both P1 and P2 are genetically uniform and highly homozygous as revealed with simple sequence repeat (SSR) markers (Vilanova *et al*., 2010).

Growing conditions

Plantlets of all materials were transplanted on 6 May, 2009, to an open field plot (sandy loamy soil) on the campus of the Universidad Politécnica de Valencia, Valencia, Spain (GPS coordinates of the field plot: latitude, 39°28′55″N; longitude, 0°20′11″W) in a completely randomised design. Plants were spaced 1.2 m between rows and 1.0 m apart within the row and drip irrigated. Fertilisation was applied with drip irrigation throughout the growing cycle and consisted of 80 g plant^{-1} of a 10N–2.2P–24.9K plus micronutrients commercial fertiliser (Hakaphos Naranja; Compo Agricultura, Barcelona, Spain). Standard horticultural practices for eggplant production in the Mediterranean coastal area of Spain were followed.

Morphological characterisation

Morphological traits were measured in individual plants using 16 primary descriptors developed by the Eggplant Genetic Resources Network (EGGNET) (Table 1; Prohens *et al*., 2005; van der Weerden & Barendse, 2007). For leaf, flower and fruit traits, five measurements were taken for each individual plant. The fruit skin primary (i.e. predominant) colour was measured in the CIELAB 1976 colour coordinates L* (0 = black; 100 = white), a* (positive values = red; negative values = green) and b* (positive values = yellow; negative values = blue), using a Minolta CR-300 chroma-meter (Minolta Co. Ltd., Osaka, Japan). Fruit flesh browning was measured as $DW_{10} - DW_0$, where DW_{10} and DW_0 are, respectively, the distances to pure white colour (L* = 100; a* = 0; b* = 0) of the fruit flesh colour measured at 0 min and at 10 min after the fruit were transversally cut with a sharp knife. Values of DW were calculated as $DW = [(100 - L^*)^2 + a^{*2} + b^{*2})]^{0.5}$ (Prohens *et al*., 2007). Fruit skin primary colour and flesh browning were measured in five fruits per plant. The measurement of the fruit flesh colour was made at the midpoint between the centre of the fruit and the pericarp.

Phenolic acid conjugate analysis

Fruit (three to five) of individual plants were harvested and brought to the laboratory, where they were washed, peeled, and a 2-cm wide longitudinal section from stem to blossom end was cut from the middle of the fruit. The excised tissue was frozen in liquid N_2 and lyophilised. The lyophilised tissue of the fruit from an individual plant was powdered and pooled as a single sample. The powdered samples were shipped from the Universitat Politècnica de València (Spain) to the USDA Beltsville Agricultural Research Center (USA) using a courier service. After being received, samples were stored at −80°C until analysed.

Subsamples of the lyophilised powdered fruit tissue (0.2 g) were extracted by vigorous stirring for 15 min at room temperature in 10 mL of methanol–water, 4:1, in a 15-mL plastic centrifuge tube that was sealed after flushing with N_2. The tube was then centrifuged at 4000 *g* for 5 min, the first extract was decanted, and the process

Table 1 Morphological traits evaluated and their description. Further details for the measurement of plant traits can be obtained from Prohens *et al*. (2005) and van der Weerden & Barendse (2007)

Trait	Code	Units/Description
Plant height	P-Height	cm
Angle between main branches	S-Angle	Sexagesimal degrees
Shoot apex anthocyanins intensity	A-Anthocyanins	0 = absent; 9 = very strong
Stem anthocyanins intensity	S-Anthocyanins	0 = absent; 9 = very strong
Stem prickles	S-Prickles	Number of prickles between two internodes
Leaf pedicel length	L-Pedicel	cm
Leaf blade length	L-Length	cm
Leaf blade breadth	L-Breadth	cm
Leaf blade length/breadth ratio	L-Length/Breadth	
Leaf blade lobing	L-Lobing	1 = very weak; 9 = very strong
Leaf anthocyanins intensity	L-Anthocyanins	0 = absent; 9 = very strong
Leaf prickles	L-Prickles	0 = none; 9 = very many
Leaf longest prickle length	L-PrickleLength	mm
Flowers per inflorescence	Fl-Number	
Corolla diameter	Fl-Diameter	mm
Fruit weight	Fr-Weight	g
Fruit skin L* primary colour	Fr-L*	CIELAB L* colour coordinate
Fruit skin a* primary colour	Fr-a*	CIELAB a* colour coordinate
Fruit skin b* primary colour	Fr-b*	CIELAB b* colour coordinate
Fruit browning index	Fr-Browning	Difference of distance to pure white between 10 and 0 min after fruit cut

was repeated on the same tissue sample. The first and second extracts were combined and 4 mL were passed through a Whatman polytetrafluoroethylene (PTFE) syringe filter (0.2 µm pore size). One millilitre aliquots of each filtered extract were transferred to amber high-performance liquid chromatography (HPLC) vials and the solvent evaporated under a stream of N_2 at 40°C. The residue was dissolved in 1.0 mL of water–methanol, 4:1, plus 0.02% phosphoric acid. Each vial was flushed with N_2 before it was sealed with a Teflon-lined septum cap. Samples were stored at −80°C until analysed by HPLC.

Phenolic acid conjugates in the fruit tissue extracts were separated and quantified by reverse phase high-performance liquid chromatography (RP-HPLC) in 50 µL injections onto a Luna C18(2) column (5 µm particle size, 250 mm long, 4.6 mm i.d.) from Phenomenex (Torrance, CA, USA) using an HP 1100 Series instrument with a quaternary pump, autosampler and photodiode array detector (Agilent Technologies, Palo Alto, CA). Data were analysed with Agilent ChemStation software (Revision B.03.01). The method used was a modification of that described by Whitaker & Stommel (2003). The binary gradient consisted of 0.02% H_3PO_4 in water (A) and methanol (B) as follows: 0 min, 90A:10B at 1.0 mL min^{-1}; 0–15 min, linear increase to 25% B at 1.0 mL min^{-1}; 15–25 min, linear increase to 50% B at 1.0 mL min^{-1}; 25–28 min, linear increases to 80% B and 1.2 mL min^{-1}; 28–30 min, linear increase to 100% B at 1.2 mL min^{-1}; 30–32 min, 100% B at 1.2 mL min^{-1}; 32–35 min, decrease to 10% B at 1.2 mL min^{-1} and 35–38 min, 10% B with linear decrease to 1.0 mL min^{-1}. Relative quantification was based on absorbance at 325 nm (caffeoyl and feruloyl conjugates) and 280 nm (dihydrocaffeoyl conjugates).

Sixteen compounds were quantified, including esters and/or amides of caffeic, dihydrocaffeic and ferulic acid. With the exception of three putative feruloyl esters of quinic acid, all the compounds have been identified by a combination of LC–MS and NMR analyses in prior studies (Whitaker & Stommel, 2003; Ma *et al.*, 2011). The 16 phenolic acid conjugates were numbered according to the order of HPLC elution but were otherwise distributed in four groups on the basis of their chemical structures (Table 2) as follows: group 1 – four isomers of caffeoylquinic acid (3-*O*-*E*, 4-*O*-*E*, 5-*O*-*E* and 5-*O*-*Z*) plus 3,5-di-*O*-*E*-caffeoyquinic acid; group 2 – six hydroxycinnamoyl amides of polyamines, including *N*-caffeoylputrescine, *N*-caffeoylspermidine, *N*,*N*′-bis(dihydrocaffeoyl)spermidine, *N*-caffeoyl-*N*′-di hydrocaffeoyl-spermidine, *N*-dihydrocaffeoyl-*N*′-caffeoyl spermidine and *N*,*N*′-bis(caffeoyl)spermidine; group 3 – three isomers of feruloylquinic acid (3-*O*-*E*, 4-*O*-*E* and 5-*O*-*E*) and group 4 – two isomers of malonylcaffeoylquinic acid (3-*O*-malonyl-5-*O*-*E*-caffeoyl and 5-*O*-malonyl-4-*O*-*E*-caffeoyl).

Data analyses

For morphological data, the mean and range of each character within individual generations were obtained.

Table 2 Phenolic acid grouping based on identification or tentative identification of the 16 hydroxycinnamic acid conjugates in eggplant fruit extracts that were quantified by high-performance liquid chromatography-ultraviolet (HPLC-UV)

Phenolic Peak	Elution Time (min)	UV A_{max} (280–330 nm)	Abbreviation	Conjugate Identification
Group 1: Mono- and di-caffeoylquinic acid esters				
3	15.1	326, 296 (sh)	3-CQA	3-*O*-(*E*)-Caffeoylquinic acid
8	21.2	326, 296 (sh)	5-CQA	5-*O*-(*E*)-Caffeoylquinic acid
10	22.4	326, 296 (sh)	4-CQA	4-*O*-(*E*)-Caffeoylquinic acid
12	24.3	319	5Z-CQA	5-*O*-(*Z*)-Caffeoylquinic acid
16	28.1	328, 296 (sh)	3,5-diCQA	3,5-Di-*O*-(*E*)-caffeoylquinic acid
Group 2: Hydroxicinnamic acid – polyamine amides (HCAA)				
1	8.2	317, 292	Caff-Put	*N*-(*E*)-Caffeoylputrescine
2	12.9	318, 292	Caff-Spd	*N*-(*E*)-Caffeoylspermidine
4	16.5	280	bis-dhCaff-Spd	*N*,*N*′-bis(Dihydrocaffeoyl)spermidine
5	18.6	319, 288	Caff/dhCaff-Spd-1	*N*-Caffeoyl-*N*′-dihydrocaffeoylspermidine
6	19.7	319, 288	Caff/dhCaff-Spd-2	*N*-dihydrocaffeoyl-*N*′-caffeoylspermidine
7	20.9	319, 293	bis-Caff-Spd	*N*,*N*′-bis(caffeoyl)spermidine
Group 3: Mono-feruloylquinic acid esters				
9	21.8	328, 299 (sh)	3-FQA	3-*O*-(*E*)-Feruloylquinic acid
13	25.4	328, 299 (sh)	5-FQA	5-*O*-(*E*)-Feruloylquinic acid
15	27.1	328, 298 (sh)	4-FQA	4-*O*-(*E*)-Feruloylquinic acid
Group 4: Malonylcaffeoylquinic acid esters				
11	23.9	327, 297 (sh)	3-Mal-5-CQA	3-*O*-Malonyl-5-*O*-(*E*)-caffeoylquinic acid
14	25.6	327, 297 (sh)	5-Mal-4-CQA	5-*O*-Malonyl-4-*O*-(*E*)-caffeoylquinic acid

Data from individual plants were subjected to analysis of variance (ANOVA), so that two ANOVAs were conducted for each of the traits studied, one which included the non-segregating (parents and hybrids) generations and another which included the segregating (F2 and BC1P2) generations. The average (pooled) variance and standard deviation for each of the traits studied was obtained from the corresponding ANOVAs. Principal components analyses (PCA) were performed for standardised morphological traits using pairwise Euclidean distances among individuals.

For phenolic acids, the percentage of individual phenolic acid conjugates in each plant, and the mean, maximum and minimum values were computed and utilised to quantify phenolic acids content for each of the four groups of phenolic acid conjugates and total phenolic acids content. Mean values and ranges for the P1, P2, F1, F2 and BC1P2 generations are reported. Heritability of phenolic acids content was determined using an additive–dominance model in which midparent and genetic effects (m = midparent, [a] = additive, [d] = dominance) were estimated using Cavalli's weighted method. Parameterisation coefficients for gene effects were as follows: m = 1 for all generations; [a] = 1 for P1, −1 for P2, 0 for F1 and F2, and −1/2 for BC1P2; [d] = 0 for P1 and P2, 1 for F1 and 1/2 for F2 and BC1P2. Model goodness of fit was tested using a weighted χ^2 (joint scaling test) (Mather & Jinks, 1977). Correlation coefficients among the different groups of phenolic acids and with total phenolic acids content were calculated.

Results

Morphological characterisation

Important differences in plant and fruit morphology were found between the *S. incanum* (P1) and *S. melongena* (P2) parents. Lack of overlap in the reported range for 12 out of the 20 traits evaluated illustrates the high level of phenotypic divergence between these species (Table 3). In this respect, the *S. incanum* parent presented no anthocyanin pigmentation in the shoot apex (A-Anthocyanins), stem (S-Anthocyanins) or leaf (L-Anthocyanins), whereas the *S. melongena* parent presented anthocyanins in all these organs. P1 was prickly in both the stem (S-Prickles) and leaf (L-Prickles), whereas P2 was non-prickly (Table 3). In addition, P1 presented shorter leaf pedicel (L-Pedicel), smaller corolla diameter (Fl-Diameter), and particularly, significantly smaller fruit weight (Fr-Weight). The fruits of P1 were green, while those of P2 dark purple (Fig. 1), which resulted in higher values for L* (i.e. less black) and b* (i.e. more yellow), and lower values for a* (i.e. more green) in P1 compared with P2. Although fruit browning (Fr-Browning) scores for P1 and P2 overlapped at the very extremes of their respective low and high score distributions, the mean Fr-Browning score was more than three times higher in P1 than in P2 (Table 3).

Heterosis for morphological attributes was evident in F1 hybrid progeny from this interspecific cross. In comparison to parental lines, F1 progeny exhibited greater plant height (P-Height), leaf length (L-Length), leaf lobing (L-Lobing) and prickle length (L-PrickelLength) (Table 3 and Fig. 1). Presence of prickles (L-Prickle) in the F1 was similar to P1. The interspecific hybrid exhibited anthocyanin pigmentation in the vegetative plant parts studied, with values similar to those observed for P2. Likewise, fruit anthocyanin pigmentation (Fr-L*, Fr-a* and Fr-b*) in the hybrid was similar to that in P2. The flowers of the F1 were about the same size (Fl-Diameter) as those of P2. F1 fruit size (Fr-Weight) was skewed towards the considerably smaller fruit size characteristic of P1. Fruit browning (Fr-Browning) scores for the F1 were greater than those of either parent (Table 3).

The distribution range and accompanying variance for traits evaluated in the segregating generations (F2 and BC1P2) were generally greater than those observed in the non-segregating generations (P1, P2 and F1) (Table 3). In particular, variation was greatest for plant architecture attributes including plant height (Pl-Height) and angle between main branches (S-Angle) and fruit characteristics (Table 3 and Fig. 1). For the F2, mean values for plant and fruit attributes were generally intermediate between both parents, but in some case skewed towards P1 or P2, for example prickle length and flower diameter. However, for leaf lobing (L-Lobing) and fruit browning (Fr-Browning), the mean values exceeded those observed for both P1 and P2 but were similar to those of the F1. Fruit weight in the F2 ranged between 13.5 and 110.2 g, with an average value (40.8 g) much greater than that of the F1 (17.2 g) (Table 3 and Fig. 1). Approximately 25% of the F2 plants lacked prickles (10 without prickles and 41 with prickles), which is a very good fit ($\chi^2 = 0.008$; $P = 0.928$) for a 3:1 segregation coinciding with the action of a single major gene. In the case of the BC1P2, mean values for plant and fruit attributes were also generally intermediate between P2 and F1 or skewed towards the F1 or recurrent parent. For example, fruit weight in the BC1P2 ranged between 14.0 and 205.0 g, with an average value of 68.1 g intermediate to parent lines. Conversely, traits such as leaf lobing (L-Lobing) and fruit browning (Fr-Browning) were skewed towards those of the F1. Unlike P2 and F1, fruit of some BC1P2 plants lacked anthocyanins, which resulted in average Fr-L* values much greater than those of any of the parents (Table 3 and Fig. 1). Fifty percent (32 out of the 64) of the BC1P2 plants lacked prickles, which is a perfect fit for a 1:1 segregation ($\chi^2 = 0.000$; $P = 1.000$) and simple inheritance.

Table 3 Mean value, range and standard deviation (SD) of the plant traits evaluated for *Solanum incanum* (P1) and *Solanum melongena* (P2) parents, the interspecific hybrid (F1), the selfed interspecific hybrid (F2) and F1 backcross to *S. melongena* (BC1P2) generations

	Non-segregating Generations							Segregating Generations					
	P1		P2		F1			F2			BC1P2		
Trait	Mean	Range	Mean	Range	Mean	Range	SD[a]	Mean	Range	SD	Mean	Range	SD
n	6		6		6			41			64		
P-Height	112.3	93 to 141	139.5	124 to 156	185.0	175 to 197	15.1	133.3	84 to 181	26.5	155.6	86 to 212	25.0
S-Angle	90.8	65 to 120	70.8	60 to 80	82.5	75 to 90	11.9	82.3	45 to 125	18.6	77.8	55 to 115	13.3
A-Anthocyanins	0.0	0 to 0	6.7	5 to 9	5.3	3 to 7	1.2	5.3	0 to 9	1.5	5.9	3 to 9	2.5
S-Anthocyanins	0.0	0 to 0	6.3	5 to 7	4.7	3 to 5	0.8	5.4	0 to 9	2.0	5.8	3 to 9	1.9
S-Prickles	7.0	5 to 9	0.0	0 to 0	9.8	8 to 14	1.6	6.6	0 to 16	6.6	4.0	0 to 14	4.0
L-Pedicel	4.3	3.9 to 4.5	8.9	6.5 to 11.0	6.8	6.4 to 7.3	0.9	6.6	3.5 to 10.0	1.5	7.1	4.3 to 10.5	1.3
L-Length	19.5	17.5 to 20.5	19.3	16.0 to 21.1	22.0	20.5 to 23.0	1.5	18.4	13.9 to 27.0	2.2	19.2	14.5 to 23.3	2.1
L-Breadth	13.3	13.0 to 13.5	14.6	12.0 to 15.6	14.7	13.8 to 15.7	1.0	12.3	9.7 to 16.7	1.7	13.1	7.8 to 17.2	1.5
L-Length/Breadth	1.46	1.30 to 1.58	1.32	1.25 to 1.43	1.50	1.46 to 1.57	0.07	1.51	1.26 to 1.88	0.15	1.47	1.28 to 1.86	0.11
L-Lobing	3.7	3 to 5	3.3	3 to 5	7	7 to 7	0.8	6.2	3 to 7	1.1	6.5	3 to 7	0.9
L-Anthocyanins	0.0	0 to 0	6.3	5 to 7	4.7	3 to 5	0.8	4.7	0 to 9	2.1	5.4	3 to 9	1.8
L-Prickles	4.3	3 to 5	0.0	0 to 0	5	5 to 5	0.6	3.2	0 to 7	2.2	2.3	0 to 7	2.5
L-PrickleLength	4.8	4 to 8	0.0	0 to 0	9.0	8 to 11	1.0	5.2	0.0 to 10.0	3.6	3.6	0.0 to 11.0	3.8
Fl-Number	5.5	4.0 to 7.0	4.0	3.0 to 6.0	6.2	5.0 to 8.0	1.1	4.8	2.8 to 7.0	1.1	4.4	2.3 to 8.0	1.1
Fl-Diameter	32.3	28.0 to 37.5	48.4	40.0 to 60.0	47.9	43.8 to 52.5	5.0	47.8	32.5 to 60.0	6.4	51.5	41.3 to 62.5	4.5
Fr-Weight	6.5	3.8 to 8.9	195.0	158.0 to 221.5	17.2	12.9 to 20.3	14.7	40.8	13.5 to 110.2	24.2	68.1	14.0 to 205.0	36.8
Fr-L*	52.1	47.8 to 57.4	23.8	19.8 to 28.3	29.6	26.6 to 34.5	3.4	43.2	20.0 to 85.9	17.5	43.3	19.98 to 87.5	19.0
Fr-a*	−18.6	−23.3 to −15.9	5.0	3.0 to 6.6	6.3	0.4 to 9.7	2.5	4.6	−14.27 to 23.54	9.9	4.5	−16.31 to 22.06	9.3
Fr-b*	20.7	14.2 to 26.3	2.1	−1.2 to 6.2	0.4	−1.8 to 1.7	2.8	7.0	−13.1 to 22.0	9.1	6.4	−3.5 to 31.6	8.8
Fr-Browning	10.6	4.5 to 15.9	3.1	2.4 to 5.3	16.8	9.6 to 26.7	4.1	16.0	5.1 to 30.2	6.5	15.3	2.8 to 36.2	7.2

[a]Pooled values for non-segregating generations obtained from analysis of variance (ANOVA) analyses.

Figure 1 Fruit samples of *Solanum incanum* (P1; a), *Solanum melongena* (P2; b), the interspecific hybrid (F1; c), the selfed F1 (F2; d) and the F1 backcrossed to *S. melongena* (BC1P2; e). Fruits are not depicted at the same scale; the size of the grid cells is 1 cm × 1 cm.

The first and second components of the PCA performed with the morphological data accounted for 24.8% and 16.7% of the total variation, respectively. The inclusion of the third or subsequent components in the PCA did not improve the interpretations. We considered as relevant those traits having correlation values with the first or second principal component greater than 0.2 (Fig. 2). The first component was positively correlated with presence of anthocyanins in fruit (high values for Fr-a*) and vegetative plant parts (S-Anthocyanins, A-Anthocyanins, L-Anthocyanins) and with flower size (Fl-Diameter) (Fig. 2). The first and second components were negatively correlated with fruit green colour (Fr-b*) and more luminous fruits (Fr-L*). The second component was positively correlated with the presence of prickles (S-Prickles and L-Prickles) and prickle size (L-PrickleLength), as well as with the leaf length/breadth ratio (L-Length/Breadth) and lobing (L-Lobing), and fruit browning (Fr-Browning). The second component was also negatively correlated with fruit weight (Fr-Weight).

The projection of the individual plants on the morphological traits PCA plot clearly separates P1 (low

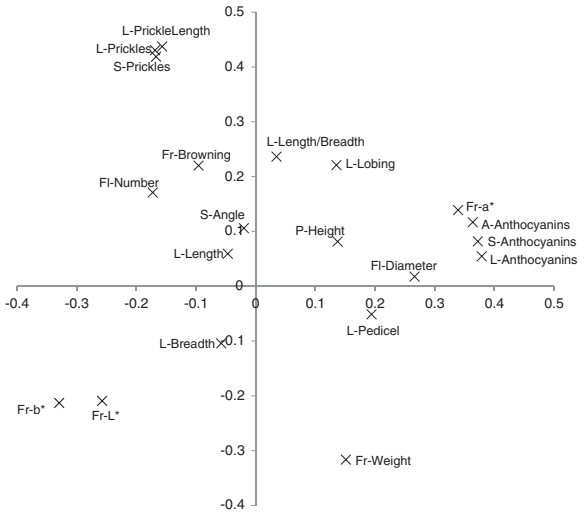

Figure 2 Principal components analysis of plant traits (see Tables 1 and 3) for *Solanum incanum* (P1) and *Solanum melongena* (P2) parents, interspecific hybrid (F1), selfing of the interspecific hybrid (F2) and backcross to *S. melongena* (BC1P2) generations. First and second components of the PCA account for 24.8% and 16.7% of the total variation, respectively.

values for the first component and intermediate values for the second component), P2 (high values for the first component and low values for the second component) and F1 (intermediate values for the first component and high values for the second component) (Fig. 3). The cluster of F1 individuals is approximately equidistant from P1 and P2 clusters. The F2 and BC1P2 individuals generally plot in the triangular area delimited by P1, P2 and F1. F2 individuals were uniformly distributed across the area bounded by the P1, P2 and F1. BC1P2 individuals were plotted most distant to P1 (Fig. 3).

Phenolic acid conjugate content

The most common phenolic compound among all the HCA conjugates identified in parental, hybrid, and F2 and BC1P2 progeny was 5-CQA, with an average value of 77.8%, and a range from 58.1% to 88.7% (Table 4). For fruit from the parental lines, 5-CQA composed a higher mean percentage of total phenolics in *S. melongena* (75.2%) compared with *S. incanum* (65.4%). All other phenolic acid conjugates were generally present in low concentrations with values below 10%. The highest average values for the minor compounds were recorded for Caff-Put (group 2; 5.5%), 3-Mal-5-CQA (group 4; 4.0%) and bis-Caff-Spd (group 2; 4.0%). However, the levels of some of these individual minor phenolic acid conjugates ranged up to 21.3%, as was the case for 3-Mal-5-CQA in one sample of *S. incanum*. Sixteen HCA esters and amides were identified across P1, P2, F1, F2 and BC1P2 progenies. Hydroxycinnamic acid conjugate profiles varied among individual plants. Presence of

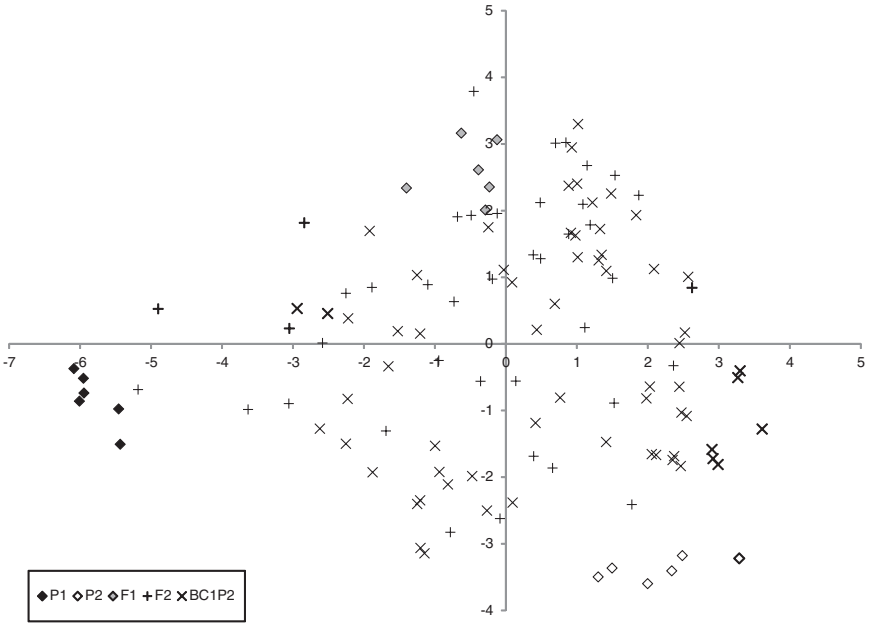

Figure 3 Similarities based on 20 morphological traits (see Tables 1 and 3) among the individual plants of the *Solanum incanum* (P1) and *Solanum melongena* (P2) parents, interspecific *S. incanum* × *S. melongena* hybrid (F1), selfing of the interspecific hybrid (F2) and backcross to *S. melongena* (BC1P2) represented on the two first components of the PCA (24.8% and 16.7% of the total variation, respectively).

Table 4 Average, maximum and minimum values for the percentage of each of the 16 hydroxycinnamic acid conjugates identified in fruit tissue extracts from 123 plants of the family constituted by *Solanum incanum* and *Solanum melongena* parent lines, the interspecific hybrid (F1), the selfed interspecific hybrid (F2) and F1 backcross to *S. melongena* (BC1P2) generations

Phenolic Acid Conjugate	Average	Min	Max
Group 1: Mono- and di-caffeoylquinic acid esters			
3-CQA	0.1	0.0	0.2
5-CQA	77.8	58.1	88.7
4-CQA	1.4	0.5	6.0
5Z-CQA	0.8	0.0	7.5
3,5-diCQA	0.5	0.0	1.9
Group 1 total	80.6	62.0	90.8
Group 2: Hydroxicinnamic acid – polyamine amides (HCAA)			
Caff-Put	5.5	0.2	21.3
Caff-Spd	0.4	0.0	1.3
bis-dhCaff-Spd	0.7	0.0	3.8
Caff/dhCaff-Spd-1	1.7	0.0	6.6
Caff/dhCaff-Spd-2	0.6	0.0	1.4
bis-Caff-Spd	4.0	0.5	12.0
Group 2 total	12.8	2.7	27.0
Group 3: Mono-feruloylquinic acid esters			
3-FQA	0.1	0.0	1.1
5-FQA	0.6	0.1	2.0
4-FQA	0.1	0.0	0.9
Group 3 total	0.8	0.1	3.0
Group 4: Malonylcaffeoylquinic acid esters			
3-Mal-5-CQA	4.0	0.1	21.3
5-Mal-4-CQA	1.9	0.1	10.4
Group 4 total	5.9	0.5	30.5

individual compounds ranged from 10 to 16 in the individual samples analysed. Seven out of 16 of the HCA conjugates (5-CQA, 4-CQA, Caff-Put, bis-Caff-Spd, 5-FQA, 3-Mal-5-CQA and 5-Mal-4-CQA) were present in all the analysed samples (Table 4). As shown in Table 4, some individuals present values of 0.0% for the contents of some of the minor hydoxycinnamic acid conjugates. However, for six of the nine compounds for which values of 0.0% are shown (5Z-CQA, 3,5-diCQA, Caff-Spd, bis-dhCaff-Spd, Caff/dhCaff-Spd-1 and Caff/dhCaff-Spd-2), we found variation for their presence/absence in all the non-segregating generations, with one or more individuals presenting values greater than zero. Among the three remaining compounds for which some individuals with 0.0% values are shown, we found 3-CQA present in all plants of both parents and absent in all plants of the F1, while 3-FQA and 4-FQA were absent in all plants of *S. melongena* (P2) and the F1, and present in some and absent in other plants of the *S. incanum* (P1) parent.

As a consequence of high 5-CQA concentrations, group 1 mono- and di-caffeoylquinic acid esters comprised the most abundant class of HCAs conjugates, with an average value of 80.6% (Table 4). With the exception of 5-CQA, the individual phenolic acids in group 1 were present at very low levels, generally below 1.5%. The hydroxycinnamic acid-polyamine amides (HCAA; group 2) ranked second in concentration, with an average value of 12.8%. Group 2 was followed by the malonylcaffeoylquinic acid esters (group 4), with an average value of 5.9%, and finally by the mono-feruloylquinic acid esters (group 3), with a very low value, 0.8% on average (Table 4).

The *S. incanum* parent (P1) exhibited much higher values than the *S. melongena* parent (P2) for all the four groups of phenolic compounds studied (Table 5 and Fig. 4). The average concentrations of groups 1, 2, 3 and 4 in P1 were 2.3, 2.1, 6.8 and 5.6 times greater, respectively, than in P2, and total phenolic levels were 2.7 times higher in P1 compared with P2. In F1 fruit, concentrations of groups 1, 2 and 3 compounds as well as total phenolics were intermediate to those in fruits of the two parents. However, abundance of the two malonylcaffeoylquinic acid isomers in group 4 was less in F1 fruit than in fruit of either parent (Table 5). The range of variation within each of the individual plants of non-segregating generations was high and, except for group 3, overlap was found between the two parents. Accordingly, comparison of the total phenolic acid conjugate content in fruit from single plants of each of the parents or the F1 hybrid revealed that for P1, P2 and F1, respectively, individuals with the highest concentration presented values that were 2.2, 3.0 and 1.7 times greater than those for individuals with the lowest concentration (Table 5).

The F2 and BC1P2 total phenolic acid conjugate concentrations were, on average, similar to those of the F1, with slightly higher levels present in the F2 compared with the BC1P2 (Table 5). As expected, the range of variation for total phenolics and for each of the four groups was generally greater in the F2 and, in particular, in the BC1P2 than in the F1 and parental generations.

On the basis of χ^2-square analysis, the variation observed in the five generations studied was adequately explained by a simple additive–dominance (m[a][d]) inheritance model (Table 5). In all cases, additive genetic variance [a] was positive. This indicates that the alleles of P1 contributed positively to high levels of total phenolics as well as to the contents of each of the four groups of phenolic acid conjugates. However, additive variance was only significant ($P < 0.05$) for group 1. The dominance parameter [d] was negative in all cases, except for group 2 and non-significant at $P < 0.05$. Addition of digenic interactions to the additive–dominance model to produce four or five parameter models provided only marginal improvements to model R^2 values for the respective groups (data not shown).

Table 5 Mean values and range (high-performance liquid chromatography peak area units at 325 nm; 280 nm for bis-dhCaff-Spd), and estimated values ± SE of genetic parameters (m = midparent, [a] = additive, [d] = dominance), χ^2 statistic and probability of χ^2 for goodness of fit to the additive–dominance genetic model proposed for the four groups of hydroxycinnamic acid conjugates identified in fruit tissue extracts for *Solanum incanum* (P1) and *Solanum melongena* (P2) parents, the interspecific hybrid (F1), the selfed interspecific hybrid (F2) and F1 backcross to *S. melongena* (BC1P2) generations

	Group 1[a]	Group 2	Group 3	Group 4	Total
Generation					
P1 ($n = 6$)					
Mean	32 456	3061	952	10 534	47 004
Range	22 307–38 380	1197–4823	278–1870	1853–18 750	28 508–61 533
P2 ($n = 6$)					
Mean	13 979	1491	139	1875	17 483
Range	7737–23 990	1124–2168	94–166	1009–3547	10 052–29 799
F1 ($n = 6$)					
Mean	22 804	2845	214	924	26 787
Range	16 781–26 849	748–4564	27–355	228–1925	18 983–31 904
F2 ($n = 41$)					
Mean	23 '320	3846	194	1894	29 254
Range	11 765–35 208	1672–9573	58–529	319–11 172	14 699–42 565
BC1P2 ($n = 64$)					
Mean	20 619	3343	198	1173	25 333
Range	7003–32 155	756–6670	48–716	108–6128	8200–40 246
Genetic parameters					
m	23 627 ± 4118	2355 ± 613	301 ± 186	5075 ± 2633	32 506 ± 6764
[a]	8794 ± 4312	829 ± 615	162 ± 187	3281 ± 2678	13 919 ± 7155
[d]	−412 ± 6343	1440 ± 1521	−114 ± 258	−4217 ± 2771	−5208 ± 9349
χ^2	0.100	0.816	1.033	0.373	0.149
Probability	0.951	0.665	0.606	0.830	0.928

[a]Group 1: mono- and di-caffeoylquinic acid esters; Group 2: hydroxicinnamic acid – polyamine amides (HCAA); Group 3: mono-feruloylquinic acid esters; Group 4: malonylcaffeoylquinic acid esters.

Correlations between the four groups of phenolic acid conjugates were highly significant ($P < 0.001$) (Table 6). Exceptions included non-significant correlations between group 2 and groups 3 and 4. Total phenolics were significantly correlated with each of the four groups, in particular with group 1, in which the correlation coefficient was greater than 0.95 (Table 6). Correlation between total phenolics content and fruit weight was negative ($r = -0.3609$; $P < 0.001$). However, when considering only the individuals of the segregating generations, the correlation coefficients were non-significant ($r = -0.3618$ for F2; $r = -0.0061$ for BC1P2) (Fig. 5). BC1P2 individuals with high total phenolic acid conjugate content and moderate fruit weights were identified (Fig. 5). The correlation between total phenolics and fruit flesh browning was low and non-significant ($P > 0.05$) when considering all individuals ($r = 0.1160$), or just those of the F2 ($r = 0.2452$) or the BC1P2 ($r = 0.0567$) generations (Fig. 6). Among the materials studied, individual plants in the F2 and BC1P2 exhibited intermediate fruit browning combined with high phenolic acid conjugate content.

Discussion

Breeding for nutraceutical quality is an increasingly important objective for vegetable improvement (Jenks & Bebeli, 2011). In the case of eggplant, the most important group of functional compounds present in the fruit is phenolics, which consist of anthocyanins in the skin and HCA derivatives, primarily chlorogenic acid, in the flesh (Whitaker & Stommel, 2003; Azuma *et al*., 2008). The concentration of HCAs present in the fruit is much higher than that of anthocyanins, and consequently the former are the major contributor to fruit antioxidant capacity (Hanson *et al*., 2006; Okmen *et al*., 2009; Singh *et al*., 2009; Lo Scalzo *et al*., 2010). As a consequence, we have initiated a breeding programme aimed at improving fruit phenolics content (Prohens *et al*., 2008; Rodríguez-Burruezo *et al*., 2008).

Although there is marked intraspecific variation for fruit phenolic content in germplasm of the cultivated eggplant, *S. melongena* (Stommel & Whitaker, 2003; Hanson *et al*., 2006; Prohens *et al*., 2007; Okmen *et al*., 2009; Akanitapichat *et al*., 2010), phenolic concentrations present in fruit of the wild eggplant ancestor *S. incanum* exceed the highest levels observed in *S. melongena*. Consequently, hybridisation with *S. incanum* affords new opportunities to improve nutritive value of cultivated eggplant (Stommel & Whitaker, 2003; Ma *et al*., 2011). Ancestral and present day selection for low fruit browning (Prohens *et al*., 2007), together with the genetic bottleneck suffered during eggplant domestication

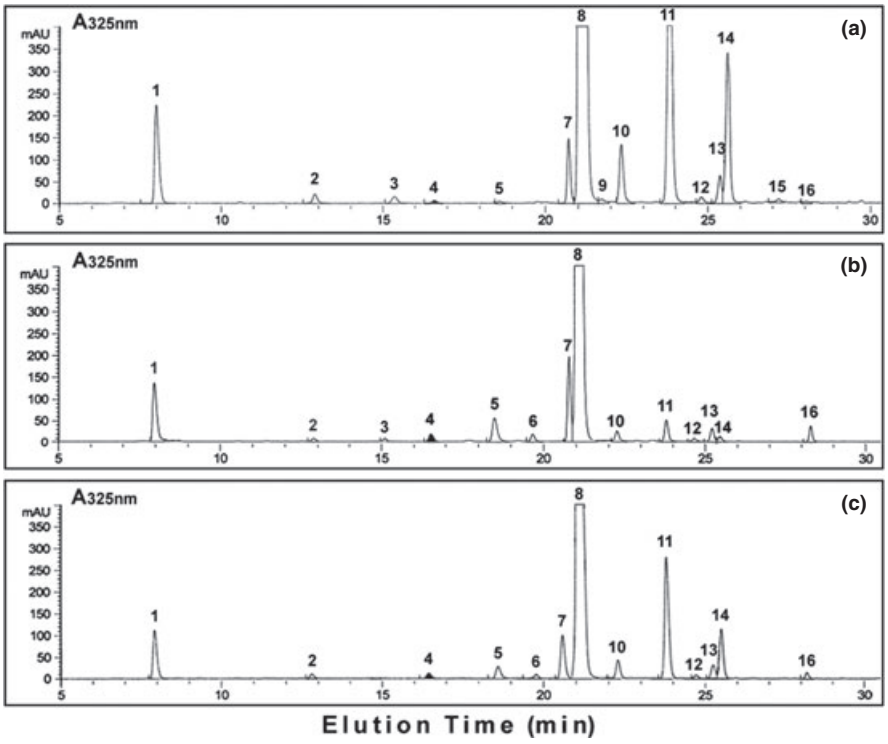

Figure 4 Representative C_{18}-high-performance liquid chromatography-ultraviolet (C_{18}-HPLC-UV) chromatograms of phenolic compounds in methanolic extracts of fruit tissues from (a) *Solanum incanum* parental (P1); (b) *Solanum melongena* parental (P2) and (c) *S. incanum* × *S. melongena* hybrid (F1) lines. Numbering of the peaks corresponds to the 16 hydroxycinnamic acid conjugates identified in Table 2. All compounds were detected at 325 nm except peak 4, bis(dihydrocaffeoyl)spermidine, which was detected at 280 nm. Note that the *S. melongena* sample (b) was injected at twice the concentration of the other two samples to better enable comparison of the relative abundance of the 16 phenolics.

Table 6 Correlations for the concentrations of the four groups of hydroxycinnamic acid conjugates identified in fruit tissue extracts of 123 plants of the family constituted by *Solanum incanum* (P1) and *Solanum melongena* (P2) parents, the interspecific hybrid (F1), the selfed interspecific hybrid (F2) and F1 backcross to *S. melongena* generations (BC1P2)

	Group 2	Group 3	Group 4	Total
Group 1	0.3263***	0.5820***	0.5115***	0.9596***
Group 2		0.0219^{ns}	-0.1032^{ns}	0.3723***
Group 3			0.7796***	0.7019***
Group 4				0.6949***

ns and asterisks (***) indicate non-significant or significant at $P < 0.001$, respectively.

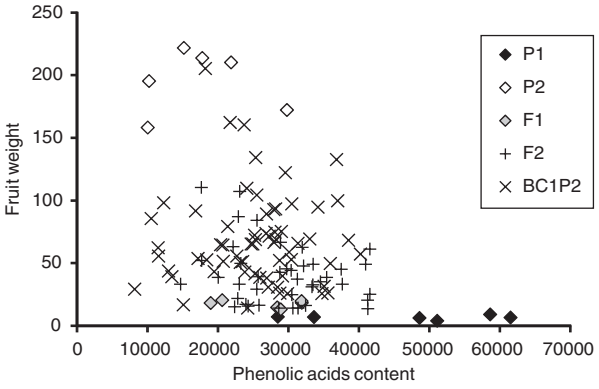

Figure 5 Relationship between total phenolic acid content (summed high-performance liquid chromatography-ultraviolet peak areas; *x*-axis) and fruit weight (in g; *y*-axis) in the individual plants of the *S. incanum* (P1), *S. melongena* (P2) parents, interspecific *S. incanum* × *S. melongena* hybrid (F1), the selfing of interspecific hybrid (F2) and backcross to *S. melongena* (BC1P2).

and evolution (Meyer *et al.*, 2012), likely resulted in the elimination of alleles in the cultivated species that contribute to high fruit phenolic content. Given that the genes involved in the biosynthetic pathway for chlorogenic acid have been identified in the *Solanaceae* (Clé *et al.*, 2008), future studies on the diversity and expression of these genes may help to elucidate key genetic factors involved in the differences between wild and cultivated species in chlorogenic acid content.

Because *S. melongena* and *S. incanum* are sexually compatible, (Lester & Hasan, 1991; Daunay, 2012), *S. incanum* is an appropriate source of variation to breed

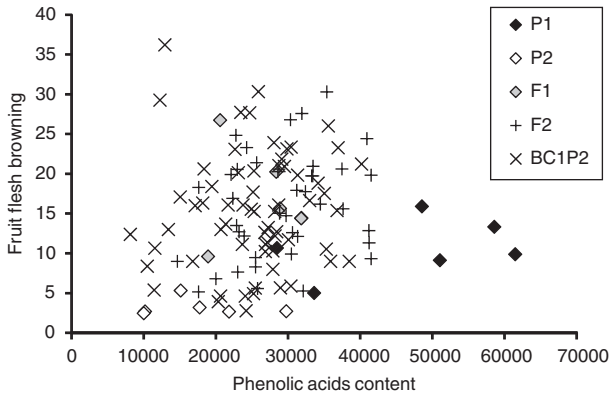

Figure 6 Relationship between total phenolic acid content (summed high-performance liquid chromatography-ultraviolet peak areas; *x*-axis) and fruit flesh browning (browning index; *y*-axis) in the individual plants of the *Solanum incanum* (P1) and *Solanum melongena* (P2) parents, interspecific *S. incanum* × *S. melongena* hybrid (F1), selfing of the interspecific hybrid (F2) and backcross to *S. melongena* (BC1P2).

for enhanced phenolic acid conjugate content in the cultivated form of the crop. However, in order to be of practical use for breeding programmes, it is important that recombinant individuals are identified in which high fruit phenolics content is combined with acceptable horticultural and fruit quality (Fita *et al*., 2010).

In this work, we describe fundamental differences between *S. melongena* and its wild ancestor *S. incanum* that contribute to horticultural quality and fruit nutritive value. Our results demonstrate that, as in other cultivated *Solanaceae* (Prohens *et al*., 2003; Paran & van der Knaap, 2007), eggplant domestication has resulted in a profound change in morphology and chemical constituents (Weese & Bohs, 2010). For example, when compared with the wild ancestor *S. incanum*, the cultivated eggplant accession we have used is typified by much larger fruit, lack of prickles and anthocyanin pigmentation in the fruit skin and vegetative plant parts. We have found that the interspecific hybrids are intermediate for many traits relative to the parental species. However, interspecific hybrids were very vigorous and heterotic for plant height. Frary *et al*. (2003) found a major quantitative trait locus (QTL) with an overdominant effect for plant height in an interspecific hybrid between *S. melongena* and *Solanum linnaeanum* Hepper & Jaeger. It has been suggested that the high vigor of the hybrids between *S. melongena* and *S. incanum* may be exploited for developing rootstocks that afford improved earliness and greater fruit yield (Gisbert *et al*., 2011). Interestingly, interspecific hybrids were pricklier than the wild species. A similar phenomenon was described in interspecific hybrids between *S. melongena* and *Solanum macrocarpon* (Lester, 1986) and between *S. melongena* and *Solanum aethiopicum* L. (Prohens *et al*., 2012) where the parents were non-prickly, but the interspecific hybrids presented prickles. Similar to the cultivated *S. melongena*, *S. melongena* *S. incanum* interspecific hybrids were anthocyanin pigmented. Fruits of interspecific hybrids exhibited a higher degree of browning in comparison with both parental species, suggesting that increased polyphenol oxidase gene expression and/or enzyme activity occurs in the interspecific hybrid. Similar observations were noted for interspecific hybrids between *S. melongena* and *Solanum viarum* Dunal (Prabhu *et al*., 2009).

As reported in backcross generations from interspecific hybrids between *S. aethiopicum* and *S. melongena* (Prohens *et al*., 2012), segregating F2 and BC1P2 generations from interspecific hybrids between *S. incanum* and *S. melongena* were generally more variable than the non-segregating generations, affording valuable opportunities for selection in a breeding programme. For most of the traits measured, average values for the F2 generation were similar to those obtained for the F1, while for the BC1P2 attributes were often intermediate between hybrids and the *S. melongena* parent. However, some important exceptions were found. Heterosis was evident for plant height. Segregation for prickliness in F2 and BC1P2 generations suggests that one major gene controls the presence of prickles in this interspecific cross. In accord with this, Doganlar *et al*. (2002) found that one major QTL in linkage group 6 explained most of the variation found for prickliness in an interspecific cross between *S. melongena* and *S. linnaeanum*. Fruit colour values in the F2 varied from those in the F1, due partly to lack of anthocyanins (present in P2) and/or chlorophylls (present in P1), and divergent stripe patterns. This same phenomenon occurred in the BC1P2. The data suggest that several genes for fruit colour segregate in this cross and that presence of fruit anthocyanin is dominant to lack of anthocyanin (Tigchelaar *et al*., 1968; Doganlar *et al*., 2002).

Despite considerable variation present in F2 and BC1P2 progenies, PCA analysis revealed that backcross progeny were distant to *S. melongena* individuals. This suggests that additional backcross generations from selected plants will be needed in order to recover the combination of traits typical of the cultivated species. Although many traits related to domestication in eggplant are simply inherited or influenced by major QTLs, they are situated in different linkage groups (Doganlar *et al*., 2002; Frary *et al*., 2003). As a result, we expected considerable segregation in the F2 and BC1P2 generations. For traits of economic importance such as fruit weight and reduced browning, individual F2 and BC1P2 plants that combined attributes similar to those of the cultivated species were identified.

Our results confirm that *S. incanum* is a useful source of variation for phenolic acid content that may be exploited

to improve levels of these functional compounds in eggplant (Stommel & Whitaker, 2003; Ma *et al*., 2011). The most common phenolic acid identified in all samples was chlorogenic acid (5-CQA). This has important implications for the improvement of the functional quality of eggplant, as many human-health benefits have been demonstrated for 5-CQA (Sawa *et al*., 1998; Triantis *et al*., 2005; Lee & Zhu, 2006; dos Santos *et al*., 2006; Kwon *et al*., 2008; Cho *et al*., 2010; Dai & Mumper, 2010). Other minor phenolics related to 5-CQA were also detected, but their concentrations were low. Hydroxycinnamic acid amides, mostly Caff-Put and bis-Caff-Spd, and malonylcaffeoylquinic acid esters, mainly 3-Mal-5-CQA, comprised the major compounds in the groups present at lesser concentrations. Mono-feruloylquinic acid esters were the least abundant HCA conjugates. These results demonstrate that 5-CQA is preferentially accumulated in eggplant and its wild ancestor, suggesting possible functional roles for this compound such as defence against plant pests, pathogens and abiotic stresses (Bradfield & Stamp, 2004; López-Gresa *et al*., 2011). Whereas fruit phenolic acid constituent profiles were fairly similar between *S. melongena* and *S. incanum*, significant differences in concentration of constituent compounds was evident. These results illustrate opportunities to increase total phenolic acid conjugate content and modify concentrations of individual constituents depending on their potential functional value. Nonetheless, breeding for improvement in the concentration of minor compounds may prove to be difficult because the study of their inheritance is complicated, for example in some cases the non-segregating generations are not uniform for the presence/absence of these individual compounds.

Wide variation present for total phenolic acid content, as well as for each of the four subgroups described, may be attributed in part to environment as illustrated by Luthria *et al*. (2010) in a comparison of phenolic compounds in eggplants produced in conventional and organic agriculture systems. In fact, we found that even in non-segregating generations, variation for presence/absence of some minor compounds occurred, indicating that environmental effects have an important influence not only on the concentration of major compounds, but also on the presence/absence of minor phenolic compounds. This has important implications for phenotypic characterisation of fruit constituents and selection of valuable germplasm for introduction into breeding programmes. When substantive environmental effects reduce trait heritability or multiple genes influence a trait, the efficiency with which desirable genotypes can be identified is reduced. In this case, increased replication, by using clonal replicates of each individual plant of the segregating generations and/or analysing several individual fruit of each plant instead of a bulked sample, would result in lower estimates of the environmental effects.

The genetic analysis of phenolic acid conjugate content demonstrated that additive effects account for a large portion of the genetic variance in phenolic constituents and total concentration. Similar results have been reported for coffee (Ky *et al*., 1999). The highest phenolic acid conjugate content will be obtained when alleles from the wild species are present in homozygosis. Development of molecular markers linked to trait-relevant structural and regulatory genes are valuable for improvement of quantitatively inherited traits. Relevant to phenolic acid quinate esters, Comino *et al*. (2009) recently developed gene-specific markers in artichoke for a chlorogenic acid hydroxycinnamoyltransferase.

Correlations identified between groups 1 (mono- and di-caffeoylquinic acid esters), 3 (mono-feruloylquinic acid esters) and 4 (malonylcaffeoylquinic acid esters) are in accord with the interconnected biosynthetic pathways for these three groups of compounds and suggest that relatively few markers may be required for coselection of these phenolic acid conjugates. Synthesis of the feruloylquinic acid isomers in group 3 rather than the corresponding caffeoylquinic acid isomers in group 1 requires the action of a single enzyme, caffeoyl-CoA 3-O-methyltransferase (Do *et al*., 2007). Similarly, synthesis of the two malonylcaffeoylquinic acid isomers in group 4 from 5-CQA and 4-CQA in group 1 is most likely catalysed by an as yet undiscovered malonyl-CoA caffeoylquinate acyltransferase (Ma *et al*., 2011). Thus, as few as two genes could be involved in linking biosynthesis of compounds in group 1 with those in groups 3 and 4. By contrast, no correlation was indicated between the hydroxycinnamic acid amides (HCAA) of polyamines in group 2 and the quinate esters in groups 3 and 4, and only a low correlation was noted between HCAA in group 2 and the caffeoylquinates in group 1. This is not surprising in light of the requirement for putrescine or spermine as substrates for HCAA biosynthesis, as well as the involvement of a specialised set of acyltransferases that form amide linkages between the HCA and polyamine moieties in HCAA (Bassard *et al*., 2010).

Illustrative of the potential to exploit the high phenolic acid conjugate content of *S. incanum* fruit in an eggplant breeding programme, we identified plants from the F2 and BC1P2 generations with combinations of high fruit phenolic content, reduced fruit flesh browning and moderate fruit weight. Utilising a collection of cultivated eggplant, Prohens *et al*. (2007) determined that total phenolics accounted for only 16% of the total variation in fruit browning. Although a negative correlation between fruit weight and phenolic acid conjugate content was found in our F2 population, the values obtained are low

(−0.3609), which means that only 13% of the variation in phenolic compounds is explained by the variation in fruit weight. Correlations between fruit weight and phenolic content were non-significant for backcross individuals. Hence, we expect that significant progress can be made in breeding market size fruit with high phenolic acid conjugate content. Also, because *S. incanum* presents comparatively high levels of solasonine and solamargine (Fukuhara & Kubo, 1991), levels of glycoalkaloids will have to be monitored in the introgression materials. In this respect, it has been shown that eggplant lines derived from introgression of allied species with high content in glycoalkaloids presented the typical low values of cultivated eggplant (Mennella *et al*., 2010).

In summary, the results obtained indicate that *S. incanum* is a valuable source of variation for improving eggplant phenolic acid conjugate content. Wide variation found for morphological traits, phenolic content and fruit browning in segregating generations descended from this *S. melongena* × *S. incanum* interspecific cross suggests that the desirable cultivated eggplant characteristics could be recovered expeditiously in a backcrossing breeding programme.

Acknowledgements

This work was partially financed by the Ministerio de Ciencia y Tecnología (AGL2009-07257 and RF-2008-00008-00-00).

References

Akanitapichat P., Kallayanee P., Nuchklang K., Prompitakkul S. (2010) Antioxidant and hepatoprotective activities of five eggplant varieties. *Food and Chemical Toxicology*, **48**, 3017–3021.

Azuma K., Ohyama A., Ippoushi K., Ichiyanagi T., Takeuchi A., Saito T., Fukuoka H. (2008) Structures and antioxidant activity of anthocyanins in many accessions of eggplant and its related species. *Journal of Agricultural and Food Chemistry*, **56**, 10154–10159.

Bassard J.E., Ullmann P., Bernier F., Werck-Reichhart D. (2010) Phenolamides: bridging polyamines to the phenolic metabolism. *Phytochemistry*, **71**, 1808–1824.

Bradfield M., Stamp N. (2004) Effect of nighttime temperature on tomato plant defensive chemistry. *Journal of Chemical Ecology*, **30**, 1713–1721.

Cao G., Sofic E., Prior R.L. (1996) Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry*, **44**, 3426–3431.

Cho A.S., Jeon S.M., Kim M.J., Yeo J., Seo K.I., Choi M.S., Lee M.K. (2010) Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. *Food and Chemical Toxicology*, **48**, 937–943.

Clé C., Hill L.M., Niggeweg R., Martin C.R., Guisez Y., Prinsen E., Jansen M.A.K. (2008) Modulation of chlorogenic acid biosynthesis in *Solanum lycopersicum*: consequences for phenolic accumulation and UV-tolerance. *Phytochemistry*, **69**, 2149–2156.

Coman C., Rugină O.D., Socaciu C. (2012) Plant and natural compounds with antidiabetic action. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **40**, 314–325.

Comino C., Hehn A., Moglia A., Menin B., Bourgaud F., Lanteri S., Portis E. (2009) The isolation and mapping of a novel hydroxycinnamoyltransferase in the globe artichoke chlorogenic acid pathway. *BMC Plant Biology*, **9**, 30.

Dai J., Mumper R.J. (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, **15**, 7313–7352.

Daunay M.C. (2012) Eggplant. In *Handbook of Vegetables*, pp. 257–322. Eds K.V. Peter and P. Hazra. Houston, TX, USA: Studium Press.

Do C., Pollet B., Thevenin J., Sibout R., Denoue D., Barriere Y., Lapierre C., Jouanin L. (2007) Both caffeoyl coenzyme A 3-O-methyltransferase 1 and caffeic acid O-methyltransferase 1 are involved in redundant functions for lignin, flavonoids and sinapoyl malate biosynthesis in *Arabidopsis*. *Planta*, **226**, 1117–1129.

Doganlar S., Frary A., Daunay M.C., Lester R.N., Tanksley S.D. (2002) Conservation of gene function in the Solanaceae as revealed by comparative mapping of domestication traits in eggplant. *Genetics*, **161**, 1713–1726.

Fita A., Tarín N., Prohens J., Rodríguez-Burruezo A. (2010) A software tool for teaching backcross breeding simulation. *HortTechnology*, **20**, 1049–1053.

Frary A., Doganlar S., Daunay M.C., Tanksley S.D. (2003) QTL analysis of morphological traits in eggplant and implications for conservation of gene function during evolution of solanaceous species. *Theoretical and Applied Genetics*, **107**, 359–370.

Friedman M., Jürgens H.S. (2000) Effect of pH on the stability of plant phenolic compounds. *Journal of Agricultural and Food Chemistry*, **48**, 2101–2110.

Fukuhara K., Kubo I. (1991) Isolation of steroidal glycoalkaloids from *Solanum incanum* by two countercurrent chromatographic methods. *Phytochemistry*, **30**, 685–687.

Gisbert C., Prohens J., Raigón M.D., Stommel J.R., Nuez F. (2011) Eggplant relatives as sources of variation for developing new rootstocks: effects of grafting on eggplant yield and fruit apparent quality and composition. *Scientia Horticulturae*, **128**, 14–22.

Hanson P.M., Yang R.Y., Tsou S.C.S., Ledesma D., Engle L., Lee T.C. (2006) Diversity in eggplant (*Solanum melongena*) for superoxide scavenging activity, total phenolics, and ascorbic acid. *Journal of Food Composition and Analysis*, **19**, 594–600.

Jenks M.A., Bebeli P.J. (2011) *Breeding for Fruit Quality*. Hoboken, NJ, USA: John Wiley & Sons Inc..

Kwon Y.I., Apostolidis E., Shetty K. (2008) In vitro studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzimes relevant for type 2 diabetes and hypertension. *Bioresource Technology*, **99**, 2981–2988.

Ky C.L., Louarn J., Guyot B., Charrier A., Hamon S., Noirot M. (1999) Relations between and inheritance of chlorogenic acid contents in an interspecific cross between *Coffea pseudozanguebariae* and *Coffea liberica* var 'dewevrei'. *Theoretical and Applied Genetics*, **98**, 628–637.

Lee W.J., Zhu B.T. (2006) Inhibition of DNA methylation by caffeic acid and chlorogenic acid, two common catechol-containing coffee polyphenols. *Carcinogenesis*, **27**, 269–277.

Lester R.N. (1986) Taxonomy of scarlet eggplants, *Solanum aethiopicum* L. *Acta Horticulturae*, **182**, 125–132.

Lester R.N., Hasan S.M.Z. (1991) Origin and domestication of the brinjal eggplant, *Solanum melongena*, from *S. incanum*, in Africa and Asia. In *Solanaceae III: Taxonomy, Chemistry, Evolution*, pp. 369–387. Eds J.G. Hawkes, R.N. Lester, M. Nee and N. Estrada. London, UK: The Linnean Society of London.

Lo Scalzo R., Fibiani M., Mennella G., Rotino G.L., Dal Sasso M., Culici M., Spallino A., Braga P.C. (2010) Thermal treatment of eggplant (*Solanum melongena* L.) increases the antioxidant content and the inhibitory effect on human neutrophil burst. *Journal of Agricultural and Food Chemistry*, **58**, 3371–3379.

López-Gresa M.P., Torres C., Campos L., Lisón P., Rodrigo I., Bellés J.M., Conejero V. (2011) Identification of defence metabolites in tomato plants infected by the bacterial pathogen *Pseudomonas syringae*. *Environmental and Experimental Botany*, **74**, 216–228.

Luthria D., Singh A.P., Wilson T., Vorsa N., Banuelos G.S., Vinyard B.T. (2010) Influence of conventional and organic agricultural practices on the phenolic content in eggplant pulp: plant-to-plant variation. *Food Chemistry*, **121**, 406–411.

Ma C., Whitaker B.D., Kennelly E.J. (2010) New 5-O-caffeoylquinic acid derivatives in fruit of the wild eggplant relative *Solanum viarum*. *Journal of Agricultural and Food Chemistry*, **58**, 11036–11042.

Ma C., Dastmalchi K., Whitaker B.D., Kennelly E.J. (2011) Two new antioxidant malonated caffeoylquinic acid isomers in fruits of wild eggplant relatives. *Journal of Agricultural and Food Chemistry*, **59**, 9645–9651.

Mather K., Jinks J.L. (1977) *Biometrical Genetics*. London, UK: Chapman & Hall.

McDougall B., King P.J., Wu B.W., Hostomsky Z., Reinecke M.G., Robinson W.E. (1998) Dicaffeoylquinic and dicaffeoyltartaric acids are selective inhibitors of human immunodeficiency virus type 1 integrase. *Antimicrobial Agents and Chemotherapy*, **42**, 140–146.

Mennella G., Rotino G.L., Fibiani M., D'Alessandro A., Francese G., Toppino L., Cavallanti F., Acciarri N., Lo Scalzo R. (2010) Characterization of health-related compounds in eggplant (*Solanum melongena* L.) lines derived from introgression of allied species. *Journal of Agricultural and Food Chemistry*, **58**, 7597–7603.

Meyer R.S., Karol K.G., Little D.P., Nee M.H., Litt A. (2012) Phylogeographic relationships among Asian eggplants and new perspectives on eggplant domestication. *Molecular Phylogenetics and Evolution*, **63**, 685–701.

Okmen B., Sigva H.O., Mutlu S., Doganlar S., Yemenicioglu A., Frary A. (2009) Total antioxidant activity and total phenolics contents in different Turkish eggplant (*Solanum melongena* L.) cultivars. *International Journal of Food Properties*, **12**, 616–624.

Paran I., van der Knaap E. (2007) Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. *Journal of Experimental Botany*, **58**, 3841–3852.

Prabhu M., Natarajan S., Veeraragavathatham D., Pugalendhi L. (2009) The biochemical basis of shoot and fruit borer resistance in interspecific progenies of brinjal (*Solanum melongena*). *EurAsian Journal of BioSciences*, **3**, 50–57.

Prohens J., Anderson G.J., Rodríguez-Burruezo A., Nuez F. (2003) Exploiting wild species for the genetic improvement of the pepino (*Solanum muricatum*). *Journal of Applied Botany*, **77**, 21–27.

Prohens J., Blanca J.M., Nuez F. (2005) Morphological and molecular variation in a collection of eggplant from a secondary center of diversity: implications for conservation and breeding. *Journal of the American Society for Horticultural Science*, **130**, 54–63.

Prohens J., Rodríguez-Burruezo A., Raigón M.D., Nuez F. (2007) Total phenolic concentration and browning susceptibility in a collection of different varietal types and hybrids of eggplant: implications for breeding for higher nutritional quality and reduced browning. *Journal of the American Society for Horticultural Science*, **132**, 638–646.

Prohens J., Muñoz-Falcón J.E., Rodríguez-Burruezo A., Nuez F. (2008) Strategies for the breeding of eggplants with improved nutritional quality. *Acta Horticulturae*, **767**, 285–291.

Prohens J., Plazas M., Raigón M.D., Seguí-Simarro J.M., Stommel J.R., Vilanova S. (2012) Characterization of interspecific hybrids and first backcross generations from crosses between two cultivated eggplants (*Solanum melongena* and *S. aethiopicum* Kumba group) and implications for eggplant breeding. *Euphytica*, **186**, 517–538.

Queiroz C., Lopes M.L.M., Fialho E., Valente-Mesquita V.L. (2008) Polyphenol oxidase: characteristics and mechanisms of browning control. *Food Reviews International*, **24**, 361–375.

Rodríguez-Burruezo A., Prohens J., Nuez F. (2008) Performance of hybrids between local varieties of eggplant (*Solanum melongena*) and its relation to the mean of parents and to morphological and genetic distances among parents. *European Journal of Horticultural Science*, **73**, 76–83.

dos Santos M.D., Almeida M.C., Lopes N.P., de Souza G.E.P. (2006) Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. *Biological and Pharmaceutical Bulletin*, **29**, 2236–2240.

Sawa T., Nakao M., Akaike T., Ono K., Maeda H. (1998) Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: implications for the anti-tumor-promoter effects of vegetables. *Journal of Agricultural and Food Chemistry*, **47**, 397–402.

Singh A.P., Luthria D., Wilson T., Vorsa N., Singh V., Banuelos G.S., Pasakdee S. (2009) Polyphenols content and antioxidant capacity of eggplant pulp. *Food Chemistry*, **114**, 955–961.

Stommel J.R., Whitaker B.D. (2003) Phenolic acid content and composition of eggplant fruit in a germplasm core subset. *Journal of the American Society for Horticultural Science*, **128**, 704–710.

Tigchelaar E.C., Janick J., Erickson H.T. (1968) The genetics of anthocyanins coloration in eggplant (*Solanum melongena* L.). *Genetics*, **60**, 475–491.

Triantis T., Stelakis A., Dimotikali D., Papadopoulos K. (2005) Investigations on the antioxidant activity of fruit and vegetable aqueous extracts on superoxide radical anion using chemiluminescence techniques. *Analytica Chimica Acta*, **536**, 101–105.

Vilanova S., Blasco M., Hurtado M., Muñoz-Falcón J.E., Prohens J., Nuez F. (2010) Development of a linkage map of eggplant based on a *S. incanum* × *S. melongena* backcross generation. In *Advances in Genetics and Breeding of Capsicum and Eggplant*, pp. 435–439. Eds J. Prohens and A. Rodríguez-Burruezo. Valencia, Spain: Editorial Universitat Politécnica de València.

van der Weerden G.M., Barendse G.W.M. (2007) A web-searchable database developed for the EGGNET Project and applied to the Radboud University Solanaceae database. *Acta Horticulturae*, **745**, 503–506.

Weese T.L., Bohs L. (2010) Eggplant origins: out of Africa, into the Orient. *Taxon*, **59**, 49–56.

Whitaker B.D., Stommel J.R. (2003) Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. *Journal of Agricultural and Food Chemistry*, **51**, 3448–3454.

Yamakawa K., Mochizuki H. (1979) Nature and inheritance of *Fusarium* wilt resistance in eggplant cultivars and related wild *Solanum* species. *Bulletin of the Vegetable and Ornamental Crops Research Station*, **6**, 19–27.